

WHAT IS CLAIMED:

1. A method of identifying a target-binding protein that binds to a predetermined target and to a serum albumin, the method comprising:

5       providing a plurality of diverse proteins; and  
       identifying one or a subset of members of the plurality which (1) interacts with a predetermined target, other than a serum albumin, and (2) binds to a serum albumin, thereby identifying a target-binding protein that binds to a predetermined target and to a serum albumin.

10

2. The method of claim 1 further comprising evaluating the *in vivo* half life of the identified member or at least some members of the subset.

3. The method of claim 1, wherein the plurality of diverse proteins comprise  
 15       members of a display library.

4. The method of claim 3, wherein the identifying comprises screening a display library.

20       5. The method of claim 1, wherein the identifying comprises screening or selecting members of the plurality of diverse proteins that interact with the predetermined target, and then screening or selecting, from those members that interact with the predetermined target, for the one or the subset of members that also bind to serum albumin.

25

6. The method of claim 1, wherein the identifying comprises screening or selecting members of the plurality of diverse proteins that bind to the serum albumin, and then screening or selecting, from those members that bind to the serum albumin, for the one or the subset of members that also interact with the predetermined target.

30

7. The method of claim 1 wherein the serum albumin is human serum albumin.

8. The method of claim 1 wherein the predetermined target is an extracellular domain of a naturally occurring protein.

5           9. The method of claim 1 further comprising administering the identified member to a subject.

10           10. The method of claim 1 further comprising formulating the identified member or one or more members of the identified subset as a pharmaceutical composition.

11. The method of claim 1 wherein each diverse protein comprises a varied peptide of less than 30 amino acids in length.

15           12. The method of claim 11 wherein the varied peptide comprises less than 4 constant positions.

13. The method of claim 11 wherein the varied peptide comprises an intramolecular disulfide bond formed by two invariant cysteine residues.

20           14. A target-binding protein isolated by the method of claim 1 and that comprises a polypeptide that (1) interacts with a predetermined target, other than a serum albumin, and (2) binds to a serum albumin.

25           15. A method of identifying a target binding protein, the method comprising:

(a) providing a plurality of library members, each of which includes a diverse protein;

(b) identifying a subset of members of the plurality that binds to a predetermined target, other than serum albumin, or to a serum albumin;

30           (c) altering the sequence of at least one member of the subset to form an altered subset that includes a plurality of variants of the at least one member; and

(d) identifying one or a subset of members of the altered subset which binds to (1) the predetermined target if the identifying in (b) is to serum albumin or (2) the serum albumin, if the identifying in (b) is to the predetermined target, thereby identifying a target binding protein.

5

16. The method of claim 15 wherein the altering comprises comparing amino acid sequences of members of the subset, inferring at least one profile for at least some of the members, and preparing the altered library by varying positions not conserved in the at least one profile.

10

17. A method of identifying a target-binding protein that binds to a predetermined target and to a serum albumin, the method comprising:

providing an initial protein that specifically binds to a target compound;

preparing a plurality of variant proteins by altering one or more amino acid

15 positions of the initial protein; and

selecting a target-binding protein that binds to a predetermined target and to a serum albumin from the plurality of variant proteins by evaluating one or more of the variant proteins for binding to the predetermined target and for binding to the serum albumin.

20

18. The method of claim 17 wherein the one or more variant proteins are evaluated by a method that comprises contacting the one or more variant proteins to immobilized serum albumin.

25

19. The method of claim 17 wherein preparing a plurality of variant proteins comprises altering a nucleic acid sequence that encodes the initial protein.

20. The method of claim 19 wherein preparing a plurality of variant proteins comprises constructing a display library.

30

21. The method of claim 17 wherein preparing a plurality of variant proteins comprises determining for the initial protein one or more amino acid positions that are non-essential for binding to the predetermined target and varying at least one of the non-essential positions.

5

22. The method of claim 17 wherein preparing a plurality of variant proteins comprises substituting at least one aromatic amino acid into an amino acid position of the initial protein.

10

23. The method of claim 17 wherein providing the initial protein comprises screening a display library.

24. An isolated peptide that specifically binds to a target molecule other than serum albumin with a  $K_D$  of less than 1  $\mu$ M and that binds to a serum albumin.

15

25. The peptide of claim 24 wherein the peptide has a length of between 6 and 32 amino acids.

26. The peptide of claim 24 wherein the peptide binds to the serum albumin with a  $K_D$  that is greater than its  $K_D$  for the target molecule.

20

27. The peptide of claim 26 wherein the peptide binds to the serum albumin with a  $K_D$  that is at least 5 fold greater than its  $K_D$  for the target molecule.

25

28. The peptide of claim 24 wherein the peptide has a half-life in vivo of at least 30 minutes in a mouse model system.

29. The peptide of claim 24 wherein the serum albumin is human serum albumin.

30

30. The peptide of claim 24 that comprises an intra-molecular disulfide bond.

31. The peptide of claim 24 that is attached to a cytotoxic moiety.

32. The peptide of claim 24 wherein the peptide comprises at least one aromatic amino acid.

5

33. The peptide of claim 24 wherein the peptide comprises an aromatic di- or tri-peptide sequence.

34. The peptide of claim 24 wherein binding of the peptide to the target molecule and binding of the peptide to the serum albumin are mutually exclusive.

10

35. The peptide of claim 24 wherein residues of the peptide that mediate binding to the target molecule and residues that mediate binding to the serum albumin are co-extensive.

15

36. The peptide of claim 24 wherein the target molecule comprises an extracellular domain of a naturally occurring protein.

37. The peptide of claim 24 wherein the target molecule is selected from the group consisting of an integrin, CEA, VEGF-R2, and MUC1.

20

38. The peptide of claim 24 wherein the peptide and any conjugated moieties has a molecular weight of less than 4500 Daltons.

39. The peptide of claim 38 wherein the peptide and any conjugated moieties has a molecular weight of less than 3500 Daltons.

25

40. The peptide of claim 24 wherein the peptide binds to the target molecule with a  $K_D$  of less than 200 nM.

30

41. The peptide of claim 40 wherein the peptide binds to the target molecule with a  $K_D$  of less than 50 nM.

42. The peptide of claim 24 wherein the peptide binds to serum albumin with a  $K_D$  of between 50 nM and 50  $\mu$ M.

43. The peptide of claim 38 wherein the peptide binds to serum albumin with a  $K_D$  of between 50 nM and 50  $\mu$ M.

44. The peptide of claim 41 wherein the peptide binds to serum albumin with a  $K_D$  of between 50 nM and 50  $\mu$ M.

45. An isolated peptide that specifically binds to a target molecule other than a serum albumin with a  $K_D$  of less than 1  $\mu$ M and that binds to a human serum albumin with a  $K_D$  that is at least 5 fold greater than its  $K_D$  for the target molecule and that is between 50 nM and 50  $\mu$ M, wherein the peptide has a length of between 6 and 32 amino acids, and wherein the peptide and any conjugated moieties has a molecular weight of less than 4500 Daltons, and wherein the peptide has a half-life in vivo of at least 30 minutes in a mouse model system.

20

46. A pharmaceutical composition comprising the peptide of claim 24 and a pharmaceutically acceptable carrier.

47. A pharmaceutical composition comprising the peptide of claim 45 and a pharmaceutically acceptable carrier.

25

48. An isolated nucleic acid comprising a sequence that encodes a polypeptide that comprises the peptide of claim 24.

49. An isolated nucleic acid comprising a sequence that encodes a polypeptide that comprises the peptide of claim 45.

30

50. A recombinant host cell that contains the nucleic acid of claim 48 and that can produce the polypeptide encoded by said nucleic acid.

5        51. A recombinant host cell that contains the nucleic acid of claim 49 and that can produce the polypeptide encoded by said nucleic acid.

52. A method of administering target-binding protein to a subject, the method comprising: administering the pharmaceutical composition of claim 46 to a subject.

10

53. The method of claim 52 further comprising evaluating a symptom of the subject.

54. The method of claim 52 further comprising imaging the subject.

15

55. The method of claim 52 further comprising evaluating clearance of the peptide from the subject

20

56. The method of claim 52 further comprising evaluating concentration of the peptide in the subject.

57. The method of claim 52 wherein the composition is administered as part of a regular dosage regimen, and the dosages are administered at least 24 hours apart.

25

58. A method of providing an agent, the method comprising: selecting a peptide agent which has been test for ability to bind to a target molecule other than a serum albumin and for ability to bind to serum albumin, thereby providing an agent.

30

59. The method of claim 58 further comprising administering the agent to a subject.